PCR protocol of XynB, ArfB and ManA1

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In previous step, we have successfully isolated pure culture of ***Clostridium stecorarium*** and cultivated it on solid medium in our lab. Then colony PCR was conducted to amplify the genes of interest from *C. stecorarium*.

The genes of interest include **α-L-arabinofuranosidase gene (ArfB)**, **endo-1,4-beta-xylanase gene (XynB)** and **mannan endo-1,4-beta-mannosidase gene(ManA1)**, three candidates for biobleaching.

In the following PCR reactions, the genome from **E.coli (DH5α)** other than *C. stecorarium* was used as template in the negative control groups.

We picked single colony from the solid plate and diluted it in **10 μl LB liquid medium**, then used **1μl** as template for colony PCR.

The recipe of the reaction mixture is shown as below:

Template	1μl
Forward primer	1μl
Reverse primer	1μl
Taq master mix (Tiangen)	12.5μl
H2O	4.5μl
Total	20μl

According to the results of gradient PCR (data not shown), the proximal annealing temperatures for the three genes were separately determined: **53.7℃ for XynB , 51℃ for ArfB, and 53.1℃ for ManA1.**

The PCR program for gene amplification is carried out as below:

Temperature(℃)			Time(s)
XynB	ArfB	ManA1	
94	94	94	30
53.7	51	51.1	30
72	72	72	120
72	72	72	600

35 repeats

(Before PCR, the bacteria were heated at 98℃ for 20 minutes in order to be lysed fully.)

Then, the PCR products (the result is shown as below) was detected by **1% agrose** gel electrophoresis . The PCR products were consistent with the expected size according to the whole genome sequencing for ***Clostridium stercorarium* DSM8532 (Poehlein et al, 2013)**, which the fragments of **XynB, ArfB and ManA1** were respectively **1164bp, 1473bp, and 1530bp.**